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Research Article

Toxocariasis in Brazilian Children: A Case-Control Study

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Abstract

Introduction: The identification of epidemiological, clinical, behavioral, and ultrasonographic and laboratory features could aid in the diagnosis of visceral larva *migrans* (VLM) in children and adolescents in Brazil.

Methods: A case-control study was conducted in ambulatory patients aged 6 months to 16 years cared for 4 years at a pediatric infectious diseases outpatient service in Belo Horizonte, Brazil. Patients with serum ELISA (enzyme-linked immunosorbent assay) anti-*T. canis* IgG antibody titers>1:640 were assigned to the case group, and the remaining subjects were assigned to the control group. The statistical significance of univariate associations between the outcome variable and exposure (i.e., epidemiological, clinical, behavioral, laboratory and ultrasonographic findings) was assessed using Pearson's chi-square (χ^2) test or Fisher's exact test. Multiple logistic regression analyses were used to assess the independent effect of each variable on the odds of the serologic status.

Results: Thirty-seven cases and 31 controls were studied. In multiple analyses, residence in a rural area (OR = 4.23; 95% CI = 0.66-27.06), keeping a dog at home (OR = 9.71; 95% CI = 1.02-92.67), and total serum immunoglobulin E (IgE)>1,000 IU/mL (OR = 2.05; 95% CI = 0.67-6.30) were the most important explanatory variables for VLM. Age, gender, hepatomegaly, splenomegaly, serum isohemagglutinins, total serum immunoglobulinG, immunoglobulin A (IgA) and immunoglobulinM, hypereosinophilia and abdominal ultrasonographic findings were not different in cases and controls.

Conclusions: Keeping dogs at home and residing in rural areas are important epidemiological risk factors for VLM. In the presence of one or more of these risk factors, patients should undergo anti-*Toxocara* ELISA. However; with larger sample studies should be performed.

INTRODUCTION

The term visceral larva *migrans* (VLM) was first proposed by Beaver et al. in 1952 to describe three cases of chronic extreme eosinophilia, hepatomegaly, pneumonitis, fever, cough and hyperglobulinemia in pediatric patients with second-stage larvae of a nematode parasite observed in granulomatous lesions from the liver[1].

Despite the high prevalence of toxocariasis in children from Brazil and abroad, there is still a paucity of epidemiological investigations on risk factors. Because the clinical suspicion of toxocariasis is sometimes challenging and delay in the diagnosis may be associated with serious complications in the eye, brain or liver, we conducted a case-control study to identify epidemiological, clinical, and ultrasonographic and laboratory features that could aid in the diagnosis of VLM in children and adolescents.

MATERIAL AND METHODS

Patients and setting

A case-control study was conducted using data collected from 68 ambulatory patients aged 6 months to 16 years cared for 4 years at the pediatric infectious diseases outpatient service of the Training and Reference Center for Infectious and Parasitic Diseases/,Belo Horizonte Health Secretary and affiliated with the Minas Gerais Federal University (UFMG),Brazil. All patients attending the outpatient service with absolute age-adjusted eosinophilia, impaired vision or liver abscess were eligible for the study. Excluded were patients diagnosed with cardiopathy, sickle cell anemia, leukemia, diabetes mellitus, HIV infection, chronic lung disease, primary or drug-induced immunosuppression, severe asthma or atopic dermatitis, and those who declined to give written informed consent.

Epidemiological, clinical and behavioral risk factors

The following epidemiological, clinical and behavioral data were gathered from clinical records using a standardized form: the location of residence (urban *vs.* rural); In addition, the following data regarding the last 3 months before diagnosis were gathered: keeping puppies (of up to 6 months-old) and dogs at home; history of use of anti-parasitic drugs by dogs at home; close contact with sand, sandboxes or soil; geophagy; onychophagy; and impaired vision (difficulty or blurred vision, or strabismus).

Laboratory data and abdominal ultrasonographic examination

Age-adjusted reference ranges for hemogram and serum Ig values were used. The parasitological stool examination was considered positive in the presence of protozoa and/or helminthes. At abdominal ultrasonography, used to define hepatomegaly and splenomegaly. Intra-abdominal lymph node enlargement and/or hypoechoic liver nodules were also considered abnormal findings.

Serum anti-*T.canis* IgG antibody titers were determined using a commercially available enzyme-linked immunosorbent assay (ELISA), Ridascreen®Toxocara IgG (Darmstadt, Germany), which detects antibodies against *T. canis* excretory-secretory larval antigens. Patients with titers >1:640 were allocated to the case group (37) and patients with titers ≤ 1:640 were allocated to the control group (31).

Data analysis

The values of the continuous variables were grouped into clinically relevant categories for statistical analysis, according to normal range values. The statistical significance of univariate associations between the outcome variable and exposure variables was assessed using Pearson's chi-square (χ^2) test or Fisher's exact test, as appropriate. Statistical significance of univariate comparisons was set at $p<0.05$ (two-tailed). For statistically significant variables, odds ratios (OR) and 95% confidence intervals (CI) were computed as measures of effect size.Multiple logistic regression analyses were used to assess the independent effect of each variable on the odds of the serologic status.

RESULTS

Sixty-eight patients were recruited aged from 10 months to 14.6 years (mean 6.7 years, median 6.6 years). Most children (66.2%) were between 10 months and 7 years-old. Males were predominant in both the cases (56.8%) and controls (67.7%) (Table 1).

A history of other parasitosis was similar among the cases and controls. Most children had contact with sand, sandboxes or

Table 1: Univariate associations between case-control status and epidemiological and socio-demographic exposure variables.

	Controls		Cases		Total			OR
Variable	**N**	**%**	**N**	**%**	**N**	**%**	**p-value**	**(95% CI)**
Gender							0.454	0.63
Female	10	32.3	16	43.2	26	38.2		(0.20-1.89)
Male	21	67.7	21	56.8	42	61.8		
Total	31	100	37	100	68	100		
Age							0.330	0.58
≤ 5 years	11	35.5	18	48.6	29	42.6		(0.19-1.72)
≥ 6 years	20	64.5	19	51.4	39	57.4		
Total	31	100	37	100	68	100		
Keeping dog at home							0.009	
No	8	26.7	1	2.8	9	13.6		12.73
Yes	22	73.3	35	97.2	57	86.4		(1.48-108.8)
Total	30	100	36	100	66	100		
Keeping puppy at home							0.268	2.54
No	5	19.2	3	8.6	8	13.1		(0.46-15.32)
Yes	21	80.8	32	91.4	53	86.9		
Total	26	100	35	100	61	100		
History of use of antiparasitic drugs by dog at home							0.746	1.38
No	18	81.8	26	76.5	44	78.6		(0.31-6.54)
Yes	4	18.2	8	23.5	12	21.4		
Total	22	100	34	100	56	100		
Close contact with sand, sandboxes or soil							0.655	1.83
No	3	10	2	5.7	5	7.7		(0.22-17.16)
Yes	27	90	33	94.3	60	92.3		
Total	30	100	35	100	65	100		
Location of residence							0.166	
Rural	2	6.7	8	21.6	10	14.9		0.26
Urban	28	93.3	29	78.4	57	85.1		(0.03-1.51)
Total	30	100	37	100	67	100		

soil, and 97.2% kept a dog at home. Approximately two-thirds of families of cases and controls earned ≤ 1 official minimum wage per month.

As for signs, symptoms and behaviors reported by patients, parents or caregivers, fever was infrequently recorded among the cases and controls. Onychophagy and geophagyprevailed among the cases (Table 2).

At physical examination, the prevalences of clinical hepatomegaly and splenomegaly among the cases were similar. At fundoscopy, 5.9% of the cases were diagnosed with retinitis (Table 3).

Abdominal ultrasonographic findings were found in 11 cases (29.7%) and liver abscess was found in 2 cases (Table 4).

Blood leukocyte counts (median value 9,275/mm^3 and maximum value 73,000/mm^3) and median blood eosinophil counts for all patients (1,396/mm^3) were not significantly different between the cases and controls. Median and maximum serum IgE levels for the case-patients were 791 IU/mL and 2,500 IU/mL, respectively. High levels of IgE were present in 97.2% of the cases, with IgE levels >1,000 IU/mL present in 50% of the cases. High levels of serum IgG, IgA and IgM were observed in 41.7%, 10.8% and 16.2% of the cases, respectively. Parasitological stool examination was positive for *Schistosoma mansoni* (3), *Strongyloidesstercolaris* (2) and *Ascarislumbricoides* (2) (Table 4).

Table 2: Univariate associations between case-control status and signs, symptoms and behaviors reported by patients, parents or caregivers.

	Controls		Cases		Total			OR (95% CI)
Variable	**N**	**%**	**N**	**%**	**N**	**%**	**p-value**	
Onychophagy							0.332	1.68
No	18	60	17	47.2	35	53		(0.56-5.04)
Yes	12	40	19	52.8	31	47		
Total	30	100	36	100	66	100		
Geophagy							0.105	2.70
No	25	83.3	24	64.9	49	73.1		(0.74-10.38)
Yes	5	16.7	13	35.1	18	26.9		
Total	30	100	37	100	67	100		
Impaired vision							1.000	
No	29	100	36	97.3	65	98.5		_
Yes	-	-	1	2.7	1	1.5		
Total	29	100	37	100	66	100		

Table 3: Univariate associations between case-control status and findings at physical examination.

	Controls		Cases		Total			OR (95% CI)
Variable	**N**	**%**	**N**	**%**	**N**	**%**	**p-value**	
Splenomegaly							0.588	0.40
No	29	93.5	36	97.3	65	95.6		(0.01-0.12)
Yes	2	6.5	1	2.7	3	4.4		
Total	31	100	37	100	68	100		
Hepatomegaly							0.525	1.49
No	27	87.1	29	78.4	56	82.4		(0.48-4.69)
Yes	4	12.9	8	21.6	12	17.6		
Total	31	100	37	100	68	100		
Peripherallymphadenomegaly							0.326	2.18
No	28	90.3	30	81.1	58	85.3		(0.44-11.94)
Yes	3	9.7	7	18.9	10	14.7		
Total	31	100	37	100	68	100		
Fundoscopy							0.495	_
Normal	29	100	32	94.1	61	96.8		
Abnormal	-	-	2	5.9	2	3.2		
Total	29	100	34	100	63	100		

Table 4: Univariate associations between case-control status and laboratory and findings from abdominal ultrasonography results.

	Controls		Cases		Total			OR (95% CI)
Variable	**N**	**%**	**N**	**%**	**N**	**%**	**p-value**	
Blood leukocytes count							0.549	0.66
Normal	24	77.4	31	83.8	55	80.9		(0.17-2.59)
Abnormal	7	22.6	6	16.2	13	19.1		
Total	31	100	37	100	68	100		
Blood eosinophil count (age-adjusted)							0.494	1.98
Normal	6	19.4	4	10.8	10	14.7		(0.43-9.55)
Abnormal	25	80.6	33	89.2	58	85.3		
Total	31	100	37	100	68	100		
Blood eosinophil count >1,000/mm³							1.000	0.57
Normal	10	32.3	12	32.4	22	32.4		(0.20-1.62)
Abnormal	21	67.7	25	67.6	46	67.6		
Total	31	100	37	100	68	100		
Total serum IgE							0.582	2.59
Normal	2	6.9	1	2.8	3	4.6		(0.17-76.54)
Abnormal	27	93.1	35	97.2	62	95.4		
Total	29	100	36	100	65	100		
Total serumIgE > 1,000 IU/mL							0.138	2.22 (0.80- 6.18)
No	20	69	18	50	38	58.5		
Yes	9	31	18	50	27	41.5		
Total	29	100	36	100	65	100		
Total serumIgG							0.301	0.63
Normal	21	72.4	21	58.3	42	64.6		(0.15-2.61)
Abnormal	8	27.6	15	41.7	23	35.4		
Total	29	100	36	100	65	100		
Total serumIgA							0.688	1.64
Normal	27	93.1	33	89.2	60	90.9		(0.23-14.08)
Abnormal	2	6.9	4	10.8	6	9.1		
Total	29	100	37	100	66	100		
Total serumIgM							0.289	2.61
Normal	27	93.1	31	83.8	58	87.9		(0.42-20.59)
Abnormal	2	6.9	6	16.2	8	12.1		
Total	29	100	37	100	66	100		
Parasitological stool examination							0.177	0.451
Negative	18	62.1	29	78.4	47	71.2		(0.13-1.51)
Positive	11	37.9	8	21.6	19	28.8		
Total	29	100	37	100	66	100		
Hepatomegaly							1.000	0.79
No	19	95	24	96	43	95.6		(0.02-31.14)
Yes	1	5	1	4	2	4.4		
Total	20	100	25	100	45	100		
Liver abscess							0.496	_
No	31	100	35	94.6	66	97.1		
Yes	-	-	2	5.4	2	2.9		
Total	31	100	37	100	68	100		
Liver hypoechoic nodules and/or periportal lymphadenomegaly							0.405	1.76
No	25	80.6	26	70.3	51	75		(0.50-6.40)
Yes	6	19.4	11	29.7	17	25		
Total	31	100	37	100	68	100		

Following the procedures described in the Data Analysis, candidate variables for the multivariate model were location of residence, number of children in the household, keeping a dog at home, geophagy, and total serum IgE > 1,000 IU/mL. Table 6 reports the final logistic regression model for VLM, showing that residence in a rural area, keeping a dog at home, and total serum IgE>1,000 IU/mL remained as the most important explanatory variables for VLM. In the presence of all 3 factors, a serum ELISA anti-*T.canis* IgG titer>1:640 would be expected in 89.4% of pediatric patients. For children who reside in rural areas and who either keep dogs at home or have a total serum IgE>1,000 IU/mL, the expected prevalence would be 80.4% and 66.6%, respectively (Table 5).

DISCUSSION

The development of currently available immunodiagnostic tests that can accurately diagnose VLM is the last step in a series of developments in VLM that began several decades ago with the first reports linking *T. canis* infection to systemic disease in human hosts.

For symptomatic children, keeping a dog at home was associated with almost 10-fold higher odds of having a serum ELISA anti-*T. canis* IgG titer>1:640 compared to children that did not own dogs. Other variables, such as the number of children in the household, geophagy, living in a rural area and a serum IgE>1,000 IU/mL, were also candidate variables for the multivariate model. In the final model, however, residence in a rural area, keeping a dog at home, and total serum IgE>1,000 IU/mL remained as the most important explanatory variables for VLM [1]. In the presence of all 3 factors, a serum ELISA anti-*T. canis* IgG titer>1:640 would be expected in 89.4% of pediatric patients.

In line with previous case-control studies [2-8], age was not associated with VLM prevalence in our sample. As previously reported in other parts of Brazil [4,9,10], VLM was more frequently observed in children older than 5. Conversely, in industrialized countries, VLM is more prevalent among children younger than 7 [11].

More case-patients in our study were males, with a male-to-female ratio of 1.3:1. Similar results were reported by several authors [12], with male-to-female ratios ranging between 1.5:1 and 2.3:1 [5,13]. However, in accordance with most published studies [2,4,6,7,9,14-17], being of male gender did not pose a statistically significant higher risk of VLM to patients. The higher prevalence of toxocariasis in male patients could be explained partially by the tendency of boys to be less stringent than girls about personal hygiene, making them more prone to infections. Impaired vision and pathologic fundoscopic findings were not more frequent among VLM patients, but the prevalence of ocular larva *migrants* [18,19], and age of presentation were comparable to previous reports.

Adults and children residing in rural areas are at increased risk of acquiring toxocariasis [6,8]. We estimated that patients residing in rural areas had approximately 4-fold higher odds for VLM than patients residing in urban areas.

As confirmed by our study and previous investigations [6-8,15,17], children owning a pet dog are at higher risk of acquiring toxocariasis. Although other authors reported an association between owning puppies younger than 3 months old and serum anti-*T.canis* ELISA positivity [14,15], our study did not confirm any significant association between VLM and owning puppies younger than 6 months old or previous use of anti-parasitic drugs by dogs at home.

Close contact with soil, sand or sandboxes has long been implicated as a risk factor for toxocariasis [14] but was not associated with increased risk in our study or in a previous report [8]. The behavioral disorder of eating earthy substances, such as soil, clay, chalk or mud, which is frequent among children between 18 months and 3 years old, increases the likelihood of eating *T. canis* ova and has been found to be a risk factor for toxocariasis in some studies [2,11]. However, we and others [14,17,20] failed to demonstrate such an association. Additionally, studies investigating the association between onicophagy and toxocariasis have shown conflicting results [4,14]. Onicophagy, common among school-age children, is sometimes considered a correlate of geophagy, more common in infants and preschoolers.

Leukocytosis and eosinophilia were not more frequent among the cases than the controls. Among the cases, the highest blood leukocyte count was 73,000/mm^3, and 48.4% had blood eosinophil counts ranging from 1,001 to 2,000/mm^3. The association between eosinophilia and serum anti-*Toxocara* positivity has also been highly inconsistent in the literature [6,8]. Other studies using different cut-off points for eosinophilia also found significant relationships between anti-*T. canis* serology and blood eosinophil counts higher than 400/mm [3,14], 1,000/mm [3,19], 2,000/mm [3,20] and 3,000/mm [3,9]. In our study, instead of using a single cut-off point for eosinophilia, we used age-adjusted reference values but did not observe higher eosinophil counts in the cases than in the controls. We hypothesize that

Table 5: Final logistic regression model for visceral larva *migrans*.

Variable	β	S.E.	OR	95% CI	p-value
Location of residence (reference: urban)					
Rural	1.442	0.947	4.23	0.66-27.06	0.128
Keeping dog at home (reference: no)					
Yes	2.273	1.151	9.71	1.02-92.67	0.048
Total serum IgE (reference: ≤ 1,000 IU/mL)					
>1,000 IU/mL	0.718	0.573	2.05	0.67-6.30	0.210
Intercept	-2.302	1.151	-	-	0.045

β: regression coefficient; S.E.: Standard Error; OR: Odds Ratio; CI: Confidence Interval; IU: International Units Hosmer-Lemeshow goodness-of-fit test, p = 0.878

eosinophilia may have been the manifestation of underlying diseases such as asthma, atopic dermatitis or exposure to other parasitosis with a life cycle involving the lungs.

The hyperimmunoglobulinemia E is a common finding in both adults [6] and children [14] with toxocariasis. We retained serum IgE> 1,000 IU/mL in the final logistic regression model because, although not statistically significant, it was associated with a 2-fold increase in the odds of VLM in our sample. The IgA, IgG and IgM titers were not predictive of VLM in our study and in other studies [14]. Because IgG intervenes in the humoral response to *T. canis* infection, we cannot rule out a lack of statistical power to detect a significant antibody rise.

The splenomegaly [14] and the hepatomegaly are not common features of VLM, although some authors reported liver enlargement in patients with toxocariasis [14]. Using routine ultrasound scanning, we were able to detect several pathologic findings in both the cases and controls. Among the case-patients, 2 had liver abscesses and 17 had hypoechoic liver nodules, with 45.5% of the latter also diagnosed with hepatomegaly. Among the controls, 6 had periportallymphadenomegaly. No ultrasonographic finding was statistically more frequent in the cases than in the controls, but the small sample size of our study and the rare event nature of these ultrasonographic findings preclude any firm conclusions. The possibility also exists that some controls may arguably be past toxocariasis patients with liver and/or abdominal lesions in a regression phase, attenuating the differences between the cases and controls toward the null.

The definite diagnosis of toxocariasis needs the demonstration of the pathogen by biopsy. However, because this is an invasive procedure, serodiagnosis using anti-*Toxocara* ELISA is acceptable. In children, serum anti-*Toxocara* antibodies remain detectable at high titers for at least 2 to 4 years [21]. In summary, this report demonstrates that keeping dogs at home and residing in rural areas are important epidemiological risk factors for VLM. In the presence of 1 or more of these risk factors, patients should undergo anti-*Toxocara* ELISA serology. Further work is needed to define serologic markers of disease severity.

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